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# **A general model based on two mixed Weibull distributions of bacterial resistance, for fitting various shapes of inactivation curves**

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## **Abstract**

Cells of *Listeria monocytogenes* or *Salmonella enterica* serovar *typhimurium* taken from six characteristic stages of growth were submitted to an acidic stress (pH=3.3). As expected, the bacterial resistance increased from the end of the exponential phase to the late stationary phase. Moreover, the shape of the survival curves gradually evolved as the physiological states of the cells changed. A new primary model, based on a two mixed Weibull distributions of cell resistances, was proposed to describe survival curves and the change of pattern with the modifications of resistance of two assumed subpopulations. This model resulted from the simplification of a first proposed model. These models were compared to the Whiting's model. The parameters of the proposed model were stable and showed a consistent evolution according to the initial physiological state of the bacterial population. Compared to the Whiting's model, it allowed best fit and more accurate estimation of the parameters. Lastly, the parameters of the simplified model had a biological significance which facilitated their interpretation.

## **1. Introduction**

When considering the thermal or non-thermal inactivation of spores or vegetative microorganisms, the log-linear shape of bacterial survival curves is a particular case among other types of curves (11, 16, 42, 48). In the case of non-thermal inactivation caused by unfavorable environmental conditions, the shape of curves presents a more pronounced heterogeneity according to the intensity of a stress. A bacterial strain can present different shapes of survival curves. Frequently concave curves may become convex or sigmoidal when the intensity of the stress varies (6, 7, 10, 18, 23, 37, 44, 46, 47). Patterns of survival curves may also vary with the physiological state of the cells and are dependent on the phase of growth (exponential or stationary phase), but also on the conditions of adaptation before the stress (17, 24, 35).

In order to model non-thermal inactivation curves, numbers of primary models were proposed. Among these models, we can find the vitalistic models proposed by *Cole et al.* (12) (27, 38), models describing both the growth and the inactivation (25, 26, 31, 36, 39, 40), the modified Gompertz model (23, 31), the exponential model (30), log-linear with latency time (6) or/and with tail (5). These models cannot deal with all shapes of curves and most of them are based on log-linear inactivation.

Some models can describe non log linear decrease or sigmoidal inactivation curves. The Weibull model was largely used in thermal and non-thermal treatment area. It is based on the hypothesis that the resistance to stress of a population follows a Weibull distribution (13, 18, 33, 43, 44). This type of model can describe linear, concave or convex curves. It was modified and extended to sigmoidal curve in the field of heat treatment (2). The model of Baranyi and Roberts (3) and that of Geeraerd *et al.* (16) can describe linear shape with or without shoulder or tail and sigmoidal shapes (20, 21). These models, which can describe sigmoidal curves, assume that the probability of survival aims towards an asymptote when the time aims towards infinity. Although they imply no further inactivation regardless of additional treatment, and their implementation does not raise any problem for short treatment time, they seem to overestimate the survival of the population for prolonged durations.

Others models are based on the hypothesis that two subgroups having different resistance to stress, coexist in the bacterial population. Cerf proposed the first model based on this assumption and on the log-linear decrease (11). Derived from this model, the Xiong's model includes a latency time to mortality (48). These models still have the disadvantage of the log-linear decrease of the population. Moreover, the Xiong's model presents a discontinuity. The Whiting's model involves a sum of two logistic models corresponding to the two subpopulations

which are characterized by their difference in resistance to stress (46). It was used to describe the non-thermal inactivation of *Salmonella*, *Listeria monocytogenes* and *Staphylococcus aureus* in brain heart infusion broth (6, 46, 47). The main advantage of this model is to be able to describe many shapes of inactivation curves often observed in non thermal inactivation.

Despite the number of proposed models, none is sufficiently flexible to be able to reflect all changes of shapes with the intensity of the stress or with the physiological state of the cells (17). In order to partially bypass this problem, the utilization of time for four decimal reductions ( $t_{4D}$ ) became widespread (6-10, 30, 31, 45-47). The  $t_{4D}$  concept presents the advantage of reflecting the evolution of the inactivation rate with respect to the various studied physicochemical factors, regardless of the patterns of various curves which can be related to a similar strain. On the other hand, this simplification does not give any information on the shape of the curves and does not allow the bacterial survival predicting at any time of the exposure to stress.

The field of non-thermal inactivation requires a model to fill this gap. In addition to robustness, parsimony, simplicity of use, biological interpretation of parameters and derivability with respect to time (for a review see (16)), this primary model should be able to describe as many shapes of inactivation curves as possible with the following requirements:

- the complete model should allow progressive simplification in order to fit simplest shapes of curves, including the log-linear first-order kinetic,
- even when survival curves are convex for long exposure times, the number of surviving cells should tend toward zero when the time tends toward the infinite. In other words, the model should not include a lower asymptote of decimal logarithm of surviving cells,

- the parameters of the model which are dependent on environmental or physiological conditions should allow a simple secondary modeling.

The model proposed by Whiting (46) might partially meet these requirements. The purpose of this work is to develop a new primary model of inactivation and to compare it with the Whiting's model on the data acquired at varying physiological states of the population.

## **2. Materials and methods**

### **2.1. Microorganism and inoculum preparation**

The studied bacterial strains were *Salmonella enterica* serovar *typhimurium* isolated from brine (strain ADQP305 obtained from ADRIA ) and *Listeria monocytogenes* isolated from meat product (strain SOR100 obtained from SOREDAB). The strains were stored at -80°C in medium composed by BHI (BIOKAR DIAGNOSTICS) broth supplemented with 50% (v/v) glycerol. The recovery of the vegetative cells was made in 100 ml of BHI broth in 250 ml flask at 37°C and shaken at 100 rotations per minute. After 8 hrs' incubation, a transplanting, 1% (v/v), was performed in a second flask of 100 ml BHI broth. In these conditions, the growth began at the average of  $10^7$  CFU.ml<sup>-1</sup>.

To study the influence of the physiological state of bacteria on the inactivation, the cells were taken at different phases of the growth. A sample (1 ml) of culture was taken and diluted in BHI broth, in order to have a concentration close to  $10^7$  CFU.ml<sup>-1</sup>. The inactivation medium was inoculated at the level of 1% (v/v) with this suspension. Each inactivation kinetic is obtained for one inoculum preparation , and then, one culture.

## 2.2. Inactivation media and numeration of survivors

A basic BHI broth (BIOKAR DIAGNOSTICS) was appropriately modified in order to generate the stress leading to the inactivation. The broth was acidified with hydrochloric acid at pH=3.3. To avoid any change in the constituents of the modified broth by heating, it was filtered on a sterile membrane of pores of 0.22  $\mu\text{m}$  diameter (Steritop system, Millipore Corporation, Billerica, MA, U.S.A.). Then, 100 ml of this broth was dispensed sterilely in culture flasks (250 ml), which were previously sterilized by autoclaving (121.1°C 20 min). Micro-organisms were inoculated in 100 ml of modified BHI broth to approximately  $10^5$  UFC.ml<sup>-1</sup>. The inactivation flasks were put in an incubator shaker (100rpm) at 12°C.

Survivors were enumerated immediately after inoculation and at appropriate time intervals by surface-plating cultures using a Spiral Plater (WASP1, Don Whitley, Shipley, West Yorkshire, UK). If dilutions were necessary to the enumeration, 0.5 ml was taken and diluted in the same modified BHI broth as the inactivation media. 1, 2, 4 and 10 ml was taken for the last four countings. According to the conditions of inactivation, the enumeration was made after variable incubation times (24 to 72 hrs) at 37°C.

## 2.3. Tested models

### ***Model 1:***

The Whiting's model (46) is derived from the model proposed by Kamau *and al.* (22), based on the logistic model. It relies on the coexistence of two subpopulations of different resistances to stress (47):

$$N(t) = N_0 \left[ f \frac{1 + e^{-k_1 \cdot t_{lag}}}{1 + e^{-k_1 \cdot (t - t_{lag})}} + (1 - f) \frac{1 + e^{-k_2 \cdot t_{lag}}}{1 + e^{-k_2 \cdot (t - t_{lag})}} \right] \quad (1)$$

Where  $t$  is time,  $N_0$  is the initial bacterial concentration,  $f$  is the fraction of the original population in the major group,  $t_{lag}$  is the latency time to mortality or shoulder period,  $k_1$  and  $k_2$  are the inactivation rates of major and secondary populations, respectively.

### ***Model 2:***

The implementation of the Weibull model to describe bacterial resistance to thermal stress has been spread during the past decades in heat treatment fields but also in non-thermal treatment (33, 43). A reparametrization of survival Weibull model (equation (2)) was proposed and applied in these fields (28, 44).

$$N(t) = N_0 \cdot 10^{-\left(\frac{t}{\delta}\right)^p} \quad (2)$$

Where  $N$  is the number of survivor,  $N_0$  is the inoculum size,  $t$  the time,  $p$  a shape parameter and  $\delta$  the treatment time for the first decimal reduction.

In order to describe all shapes of inactivation kinetics and it was assumed that the population is composed of two groups different in their resistance to stress. The resistance of each subpopulation is assumed to follow a Weibull distribution. Then the size of the surviving population can be described by the following equation:



$$N(t) = N_0 \left( f \cdot 10^{-\left(\frac{t}{\delta_1}\right)^{p_1}} + (1-f) \cdot 10^{-\left(\frac{t}{\delta_2}\right)^{p_2}} \right) \quad (3)$$

Where indices 1 and 2 are linked to the two different subpopulations. Subpopulation 1 is more sensitive to stress than the subpopulation 2 ( $\delta_1 < \delta_2$ ).  $f$  is the fraction of t subpopulation 1 in the population.

Without mathematical transformation, the  $f$  ratio yields a problem of insufficient discrimination. The fraction  $f$  varying from 0 to 1, in order to have a more discriminating parameter, a new parameter ( $\alpha$ ) varying from negative infinity to positive infinity was introduced from a logit transformation of  $f$ :

$$\alpha = \log_{10} \left( \frac{f}{1-f} \right) \quad (4)$$

It is equivalent to:

$$f = \frac{10^\alpha}{1 + 10^\alpha} \quad (5)$$

With this transformation, a  $f$  ratio equal to 0.999999 or a  $f$  ratio equal to 0.999900 corresponds to  $\alpha$  values equal to 4 and 6 respectively. It is equivalent to a hundred fold multiplication of the subpopulation 2 initial size. After the introduction of the  $\alpha$  value, the equation (3) became:

$$N(t) = \frac{N_0}{1 + 10^a} \left[ 10^{-\left(\frac{t}{\delta_1}\right)^{p_1 + a}} + 10^{-\left(\frac{t}{\delta_2}\right)^{p_2}} \right] \quad (6)$$

### **Model 3:**

When an enumeration at low concentration was possible, the right part of the curves, corresponding to the most resistant subpopulation 2, seemed to be convex like the most sensitive subpopulation 1. It was then proposed to simplify the equation by affecting the same shape parameter to the two subpopulations. The final model was:

$$N(t) = \frac{N_0}{1 + 10^a} \left[ 10^{-\left(\frac{t}{\delta_1}\right)^{p + a}} + 10^{-\left(\frac{t}{\delta_2}\right)^p} \right] \quad (7)$$

#### **2.4. Parameter estimation, confidence intervals and model evaluation**

To describe the evolution of survival curves, the survival ( $N_i$ , CFU.ml<sup>-1</sup>) during time was expressed as follows:

$$Y_i = f(t_i, \theta) + \varepsilon_i \quad (8)$$

Where  $Y_i$  is the decimal logarithm of  $N_i$ , and  $f$  is the regression function. The vectors of parameters of models  $\theta$  were estimated by minimization of the sum of square of the residual values ( $\varepsilon_i$ ) defined by:

$$C(\theta) = \sum_{i=1}^n (Y_i - f(t_i, \theta))^2 \quad (9)$$

Where  $n$  is the number of data. The minimum  $C(\theta)$  values were computed with non-linear fitting module (NLINFIT, MATLAB 6.1, Optimization Toolbox, The Math-works).

The fit of the models was compared using the Akaike Information Criterion (AIC) (1) :

$$AIC = -2.\ell(\theta) + 2.p \quad (10)$$

Where  $p$  is the number of parameters of the model, and  $\ell(\theta)$  is the log-likelihood. In the case of Gaussian observations, the least square estimator of  $\theta$  is also the maximum likelihood estimator (19). The logarithm of the likelihood is generally used instead of the likelihood itself and it is defined as follows:

$$\ell(\theta) = -\frac{n}{2}.\log(2\pi) - \frac{1}{2}\sum_{i=1}^n \left[ \log(Var(\varepsilon_i)) + \frac{(Y_i - f(t_i, \theta))^2}{Var(\varepsilon_i)} \right] \quad (11)$$

Herein  $n$  is the number of points of the curve, and  $Var(\varepsilon_i)$  the variance of the residual  $\varepsilon_i$ .

The AIC criterion permits to compare models by taking both the goodness of fit and the parsimony into account (1, 29). A great number of parameters or a poor quality of fit (small log-likelihood value) corresponds to a high value of AIC. Then best models yield smallest AIC values.

The likelihood ratio test was used to test whether  $p_1$  and  $p_2$  parameters of the model (2) are identical, in order to check the validity of the model (3) (19). Let  $\theta_H$  be the estimation of  $\theta$  under the constraint of the equality of the parameters, equivalent to the estimation of the model (3) parameters and  $\theta_A$  be the unconstraint estimation of  $\theta$ , equivalent to the estimation of the model (2) parameters. Let :

$$S_L = -2[\ell(\theta_H) - \ell(\theta_A)] \quad (12)$$

be the statistic test. If  $p_1$  and  $p_2$  parameters are equal, the  $S_L$  value will be small. When  $n$  tends to infinity, it can be shown that the limiting distribution of  $S_L$  is a  $\chi^2$  distributed with one degree of freedom (difference in dimensionality of  $\theta_A$  and  $\theta_H$ ).

### 3. Results

#### 3.1. Influence of the physiological state of cells on the pattern of survival curves

The cells which were submitted to an acidic stress at pH 3.3 were taken at the six following characteristic phases of the growth (figure1):

- i. Beginning of the exponential phase (1.67 hours after inoculation, or 0.10 of O.D.<sub>.600nm</sub> for *Listeria monocytogenes* and 0.15 for *Salmonella typhimurium*).
- ii. Middle of the exponential phase (3.33 hours after inoculation, or 0.20 of O.D.<sub>.600nm</sub> for *Listeria monocytogenes* and 0.60 for *Salmonella typhimurium*).
- iii. End of the exponential phase (5 hours after inoculation, or 0.55 of O.D.<sub>.600nm</sub> for *Listeria monocytogenes* and 0.70 for *Salmonella typhimurium*).
- iv. Deceleration phase of the growth (6.67 hours after inoculation, or 0.70 of O.D.<sub>.600nm</sub> for *Listeria monocytogenes* and 0.75 for *Salmonella typhimurium*).
- v. Early stationary phase (12 hours after inoculation, or 0.80 of O.D.<sub>.600nm</sub> for *Listeria monocytogenes* and 0.85 for *Salmonella typhimurium*).
- vi. Late stationary phase (17 hours after inoculation, or 0.85 of O.D.<sub>.600nm</sub> for *Listeria monocytogenes* and 0.80 for *Salmonella typhimurium*).

The survival curves of *Salmonella typhimurium* show a continuous and progressive evolution from a biphasic shape to a simple concave shape whether cells are taken from early or late stages of growth (figure 2). This evolution seems to correspond to the gradual disappearance of a sensitive subpopulation. In the case of *Listeria monocytogenes* (figure 3), the initial presence of two subpopulations is less clear, but a drastic increase of the general resistance of bacteria can be observed: while the elimination of the total population seems to be reached within around 3

days for cells of the early stage of growth (figure 3, i), it takes more than 30 days before the same level of inactivation can be reached when cells are taken at the late stationary phase (figure 3, vi)

### 3.2. Quality of fit

We compared the Whiting's model (1) and the two new proposed models for describing the survival of bacteria at various times of incubation of subcultures (figure 2, figure 3).

The model (2) which includes one more parameter than the other ones, had, as it could be expected, the best fit on the data according to the minimum sum of squares  $C(\theta)$  for sixteen cases among twenty observed curves (table 1). However, this model is the worst according to the AIC criterion which takes both the fit and the parsimony into account. In most cases, the Whiting's model (1) and the simplified model (3) showed quite equivalent goodness of fit according to the AIC criterion. The double Weibull simplified model (3) yielded a slight tendency of better fit with fourteen smaller AIC criterions for the twenty observed kinetics. In some cases, there were great differences between the two AIC criterions in favor of the model 3 (table 1 and figure 2 vi for *Salmonella*, and figure 3 iii and vi for *Listeria*). In these cases the model (3) presented a very small AIC value compared to the model (1), the difference could reach about twenty units for *Salmonella* and seventy units for *Listeria*.

It could also be noted that the confidence intervals related to the Whiting's model (1) were larger, especially for the  $f$  value (results not shown). The confidence intervals of the estimated parameters and the AIC of the model (3) were smaller, showing a better estimation of parameters and better compromise between the goodness of fit and the parsimony.

The hypothesis of equality between the  $p_1$  and  $p_2$  of the model (2) was at the origin of the model (3). If the likelihood ratio test value ( $S_L$ ) is lower than the value of the  $\chi^2$  with 1 degrees of

freedom for the significance level of 0.05, the tested hypothesis cannot be rejected. The hypothesis was not rejected by the likelihood ratio test in fifteen out of twenty cases (table 1). One of the two repetitions was not validated for the cases from iii to vi of *Listeria monocytogenes*. The AIC criterion was favorable to the simplification of the double Weibull model. This simplification allowed removing one parameter while keeping nearly the same goodness of fit.

### 3.3. Effect of the physiological state on the estimated parameters of models

According to the Whiting's model (1) and the two studied species, the estimated  $f$  ratio fell from 100% to 30% after 300 minutes of incubation of the subculture, while estimated values of  $t_{lag}$  increased from an average of 15 hrs to more than 100 hrs and seemed then to stabilize at an average of 720 min (model (1) figure 4 and figure 5). Regarding *Salmonella*, the estimated  $t_{lag}$  values were very different between the last two replicates but the associated confidence intervals were wide. For the two studied species,  $k_1$  value fell to a value close to  $k_2$  which was approximately constant with an average value of  $0.01 \text{ h}^{-1}$ .

The  $\delta_2$  estimated values of the model (2) did not seem to change with the duration of the incubation of the subculture, for *Salmonella*, this value was close to 200 hrs. For the two species, the  $\delta_1$  values increased from 15 hrs to more than 100 hrs and tended toward  $\delta_2$  values. The values of  $\alpha$  decreased from 4 or 5 respectively for *Salmonella* and *Listeria* to 1, equivalent to  $f = 99.990\%$ ,  $99.999\%$  and  $90.909\%$  respectively. However the profiles of the evolution of the parameters were quite different. For *Salmonella*, this parameter was equal to 4 for an incubation of the subculture less than 300 minutes, after this time the  $\alpha$  value decreased to 1. Contrary to *Salmonella*, the parameter  $\alpha$  decreased quickly from 5.3 to 2 for an incubation duration lower

than 400 minutes while for *Listeria*, it continued to have a slow decline to 1 until after this time. The  $p_1$  and  $p_2$  values were very variable and chaotic, between 1 and 31.

Regarding the double Weibull simplified model (3), the evolutions of the  $\delta$ 's parameters were similar to that observed from the model (2). The only differences of behavior between these two models concerned the  $\alpha$  and  $p$  parameters. As opposed to the model (2), the rate of decrease of  $\alpha$  estimates from the model (3) was less variable for the two species. The  $p$  value was relatively stable excepted for cells resulting from the late stationary phase, which had a slight tendency to increase for *Salmonella*. The median of the  $p$  value was close to 2 for the two species.

#### 4. Discussion

As expected, the resistance of bacterial populations to stress increased with the approach of the stationary phase. Such an increase of the resistance cultures results from the initiation of depending mechanisms on physicochemical factors of the bacterial environment but also on the reduction of the metabolic activity of cells (4, 24, 32, 41). A clear change of shape in the inactivation kinetic curves could be noted.

The evolution of the parameter values related to models was directly linked to the augmentation of the resistance. The inactivation rate ( $k_1$ ) or the first decimal reduction time ( $\delta_1$ ) of the most sensitive population increased, while the rate of inactivation of the resistant population kept unchanged in a wide range. The decrease of the ratio  $f$ , or its logit  $\alpha$ , corresponding to an augmentation of the ratio of more resistant cells, was at the origin of this change. The Whiting's model had five parameters, four among which, ( $k_1$ ,  $k_2$ ,  $f$ ,  $t_{lag}$ ) characterize the evolution of the resistance of the overall population with respect to the duration of subculture

incubation. On the other hand, the double Weibull simplified model (3) had also five parameters, but only 3 parameters ( $\delta_1$ ,  $\delta_2$ ,  $\alpha$ ) were physiological state of cells and of environmental conditions.

Furthermore, the models (1) and (3) presented equivalent quality of fit except in the cases  $f$  for *Salmonella* (figure 2, table 1) and  $f$  and  $c$  for *Listeria* (figure 3, table 1). In these cases, the shape of the kinetics was biphasic non-linear. The Whiting's model is based on a linear decrease of the subpopulation after latency to mortality. It was unable to describe the concave decrease observed in these particularly cases, which explains the bad value of the AIC value related to the Whiting's model (1). The double Weibull simplified model (3) is more flexible and could describe the biphasic non-linear shape ( $p$  parameter higher than 1) as well as the biphasic linear case ( $p$  parameter equal to 1).

With closer confidence intervals, the double Weibull model described the adaptation of cells better than the model of Whiting. For the first four durations of incubation corresponding to the exponential phase of the subculture, the time necessary for the first decimal reduction  $\delta_1$  value increased and was stabilized to the  $\delta_2$  value during the stationary growth phase of the subculture (figure 4 and figure 5). This evolution testified to the adaptation of the cells from the most sensitive subpopulation 1, whose resistance to stress tended gradually towards the resistance of subpopulation 2. The resistance of the subpopulation 2 is stable. The  $\alpha$  value decreased with the promotion of the subculture along various stages of growth, pointing out the increase of the ratio of the resistant subpopulation to stress. The increase of the resistance to stress was well described by the combined evolutions of all parameters which pointed out the progressive passage of the resistance from a sensitive to a resistant grade. The population 1 assumed by the model is the most sensitive and had not activated or slightly activated the mechanisms of resistance. The population 2 corresponds to the most resistant cells having a restricted metabolic activity and



having developed the mechanisms of resistance. When the resistance is minimal or maximal, a single population should be observed corresponding to the subpopulations 1 and 2 respectively. Then, the resistance to stress should follow a simple Weibull distribution.

One advantage of the parameterization and the simplification of the model (3) is that all parameters can be graphically interpreted (figure 6):

- $N_0$  is the initial size of the population,
- $\delta$  are the time of the first logarithm decline for the two subpopulations,
- $\alpha$  is defined as the logit of  $f$ . It is equivalent to:  $\alpha = \log_{10} \left( \frac{N_{0_1}}{N_{0_2}} \right)$

The  $\alpha$  value then is close to the graphic difference between  $\log_{10}(N_0)$  and the logarithm of the population size where the inflexion is observed.

- $p$  represents the shape of the curve (see below).

In theory, the  $\alpha$  value can be equal to all real numbers. In practice, note that no inflection point can be obviously graphically observed for negative  $\alpha$  value. This is also the case if its value is higher than the difference between the  $\log_{10}(N_0)$  and the decimal logarithm of detection limit of the technique of enumeration. In this particular cases, the  $\alpha$  value is not observed and then cannot be estimated.

The double Weibull simplified model allows to fit most of the shapes of inactivation curves (figure 7). With two populations, it can describe:

- in the general case: biphasic shape with non linear decrease (a), note that it cannot be described by the other models used in the bacterial inactivation field in constant conditions of stress.

- if  $\delta_2$  tends toward infinity: sigmoidal shape (b).

- if  $p=1$ : biphasic shape (f).

- if  $\delta_2$  tends toward infinity and  $p=1$ : linear with tail (g).

The simplification of the double Weibull model to a simple one can be obtained by negative value of  $\alpha$ , or  $\alpha > \log_{10}(N_0 / \text{detection limit})$ , or equality between the two  $\delta$  values. Then, it permits the fit of:

- if  $p=1$ : linear shape (d).

- if  $p>1$ : concave shape (c).

- if  $p<1$ : convex shape (e).

This work would require further research to allow the use of the double Weibull model in the non thermal inactivation field. The evolution of  $p$  according to the environmental factors and the physiological state of cells requires a special study. For the thermal treatment, in some cases it can be considered as constant (14, 15, 34, 43). The advantage of the model of Weibull is to have a great flexibility on account of a strong correlation between the scale ( $\delta$ ) and the shape ( $p$ ) parameters. If the  $p$  value is estimated as a constant value among different conditions of stress, the  $\delta$  parameter is able to balance this constraint to give a good quality of fit of the model on the data. If this phenomenon could be confirmed in the field of non thermal inactivation, the double Weibull model might be a convenient model describing the kinetics as a function of the physiological state of the cells and the conditions of stress with only three parameters. Indeed, the

parameters  $\delta$ 's might evolve according to the intensity of stress, and the parameters  $\delta_I$  and  $\alpha$  according to the physiological state of the treated cells as was shown.

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Table 1 Minimum  $C(\theta)$  and AIC criterions for the different survival curves of Salmonella typhimurium and Listeria monocytogenes. (1) the Whiting's model, (2) the double Weibull model and (3) the double Weibull simplified model. The  $S_L$  values are related to the simplification of the model (2) into the model (3), Bold  $S_L$  values represent the rejection of the simplification. For each case, bold values represent the best value of  $C(\theta)$  or AIC criterions.

Figure 1 Evolution of the population size ( $\circ$ , cfu.ml<sup>-1</sup>), of optical density ( $\diamond$ , DO 600 nm) and pH (\*) during growth preceding the inactivation of *Listeria monocytogenes* (a) and *Salmonella typhimurium* (b). The characteristic phases of growth are (i) the beginning of the exponential phase, (ii) middle of the exponential phase, (iii) end of the exponential phase, (iv) deceleration of the exponential phase, (v) early stationary phase, (vi) late stationary phase

Figure 2 Evolution of the shape of survival curves of *Salmonella typhimurium* and the fitted curves of the models. Whiting's model (1) (—), model (2) (- -), simplified model (3) (— —). The observed data are represented by points. The title (i), (ii), (iii), (iv), (v) and (vi) are linked to the characteristic phases of growth from which came the inoculum used for inactivation kinetics (see text and figure 1). The subtitle 1 or 2 is linked to the repetitions.

Figure 3 Evolution of the shape of survival curves of *Listeria monocytogenes* and the fitted curves of the models Whiting's model (1) (—), model (2) (- -), simplified model (3) (— —). The observed data are represented by points. The title (i), (ii), (iii), (iv), (v) and (vi) are linked to the characteristic phases of growth from which came the inoculum used for inactivation kinetics (see text and figure 1). The subtitle 1 or 2 is linked to the repetitions.

Figure 4 Evolution of the estimated parameters versus the time of incubation of subculture for *Salmonella typhimurium*. (1) Whiting's model, (2) the double Weibull model and (3) the double Weibull simplified model. The  $k_1$ ,  $f$ ,  $t_{lag}$ ,  $\delta_1$ ,  $\alpha$ ,  $p$  and  $p_1$  values are represented by points, and The  $k_2$ ,  $\delta_2$  and  $p_2$  values are represented by circles.

Figure 5 Evolution of the estimated parameters versus the time of incubation of subculture for *Listeria monocytogenes*. (1) the Whiting's model, (2) the double Weibull model and (3) the

double Weibull simplified model. The  $k_1, f, t_{lag}, \delta_1, \alpha, p$  and  $p_1$  values are represented by points, and The  $k_2, \delta_2$  and  $p_2$  values are represented by circles.

Figure 6 Diagram of survival model based on the double Weibull distribution of the resistance. (—) microbial population; (- -) subpopulation 1; (— —) subpopulation 2. With subpopulation 1 representing bacteria the most sensitive to the stress, and the subpopulation 2 representing on the contrary cells the most resistant.

Figure 7 Different shape of inactivation curves: biphasic with non linear decrease (a), sigmoidal (b), concave (c), linear (d), convex (e), biphasic (f), linear with tail (g)

Table 1

Organism	Characteristic stages of the culture-repetitions (see text)	i-1	ii-1	iii-1	iii-2	iv-1	iv-2	v-1	v-2	vi-1	vi-2
	Time of incubation of subculture (min)	100	200	300	300	400	400	720	720	1020	1020
<i>Salmonella typhimurium</i>	n	13	16	19	20	24	22	38	33	31	34
	$C(\theta)^{(1)}$	0.119	0.742	1.796	1.131	1.054	0.822	0.825	1.246	2.067	1.510
	$C(\theta)^{(2)}$	<b>0.082</b>	<b>0.677</b>	<b>1.602</b>	<b>0.984</b>	<b>1.069</b>	<b>0.784</b>	<b>0.772</b>	<b>1.039</b>	<b>1.018</b>	<b>1.085</b>
	$C(\theta)^{(3)}$	0.089	0.751	1.602	1.027	1.115	0.784	0.874	1.108	1.122	1.085
	$S_L$	0.92	1.65	0.00	0.03	1.00	0.02	<b>4.73</b>	2.11	3.00	0.00
	AIC <sup>(1)</sup>	-14.11	<b>6.31</b>	19.13	11.33	<b>3.12</b>	2.14	<b>-27.67</b>	-2.48	14.04	2.61
	AIC <sup>(2)</sup>	-16.85	6.84	18.96	9.38	5.45	1.09	-28.22	-8.44	-5.91	-8.62
	AIC <sup>(3)</sup>	<b>-17.93</b>	6.49	<b>16.96</b>	<b>7.41</b>	4.46	<b>-0.90</b>	-25.49	<b>-8.33</b>	<b>-4.91</b>	<b>-10.62</b>
<i>Listeria monocytogenes</i>	n	11	16	33	29	30	48	32	41	36	46
	$C(\theta)^{(1)}$	<b>0.452</b>	1.076	7.618	0.689	1.452	3.244	0.560	1.592	1.069	2.655
	$C(\theta)^{(2)}$	0.455	<b>1.045</b>	<b>0.887</b>	<b>0.549</b>	<b>1.196</b>	<b>2.324</b>	<b>0.542</b>	<b>0.361</b>	<b>1.057</b>	<b>0.812</b>
	$C(\theta)^{(3)}$	0.460	1.046	0.887	0.552	1.444	2.326	0.571	0.591	1.059	0.935
	$S_L$	0.14	0.01	0.01	<b>6.58</b>	<b>5.65</b>	0.03	1.72	<b>20.15</b>	0.04	<b>6.50</b>
	AIC <sup>(1)</sup>	<b>6.15</b>	12.25	55.29	-14.13	4.31	18.90	<b>-28.65</b>	-4.84	-14.44	9.35
	AIC <sup>(2)</sup>	8.22	13.78	-13.69	-20.70	<b>0.48</b>	2.89	-27.70	-65.63	-12.82	-43.17
	AIC <sup>(3)</sup>	6.35	<b>11.79</b>	<b>-15.69</b>	<b>-22.54</b>	4.13	<b>0.92</b>	-27.99	<b>-47.48</b>	<b>-14.78</b>	<b>-38.67</b>



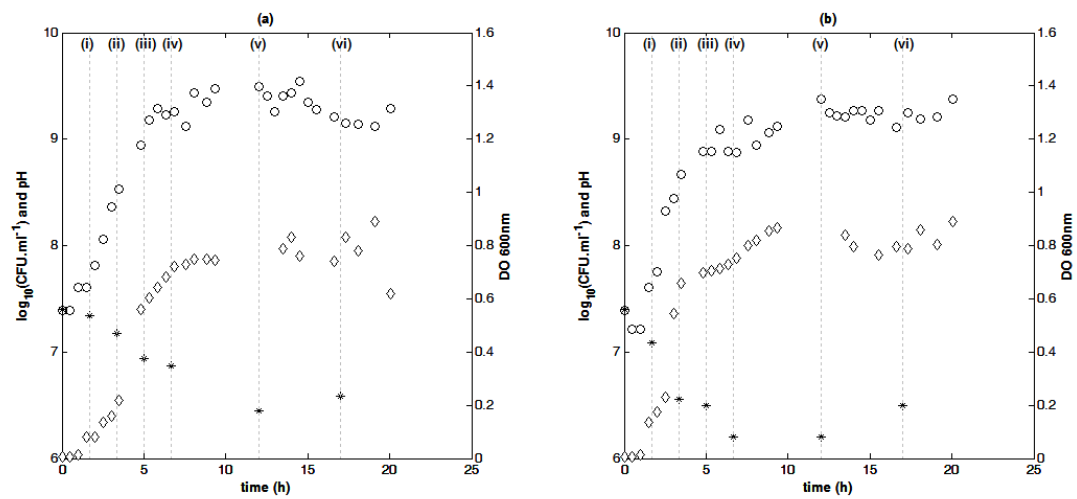


Figure 1



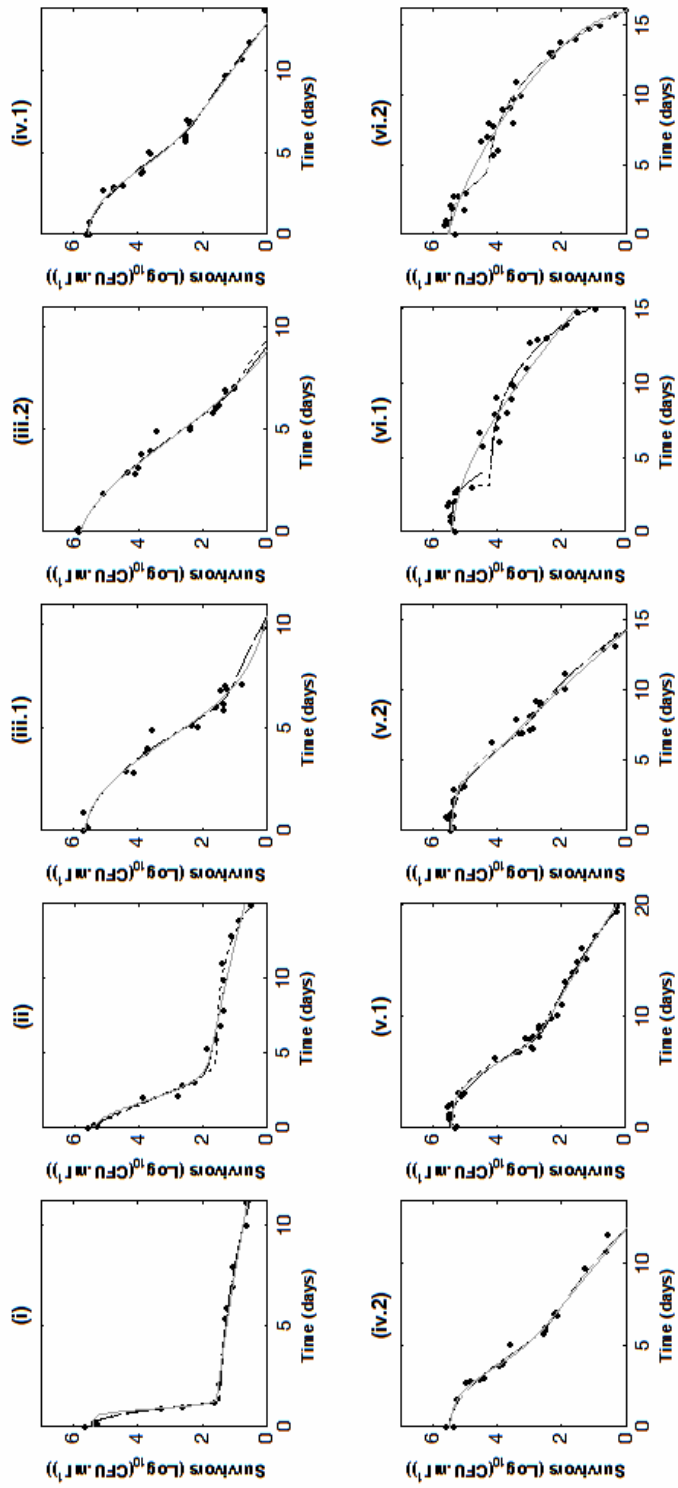


Figure 2

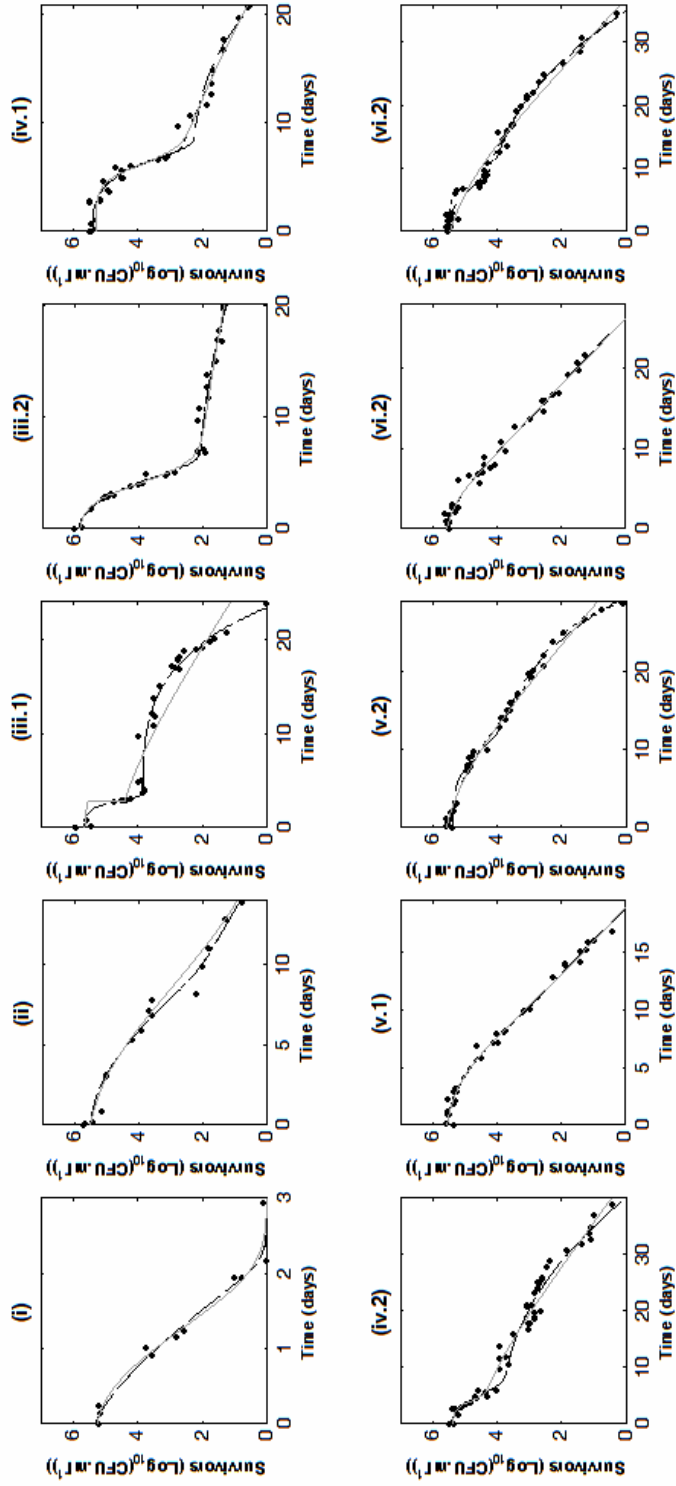


Figure 3

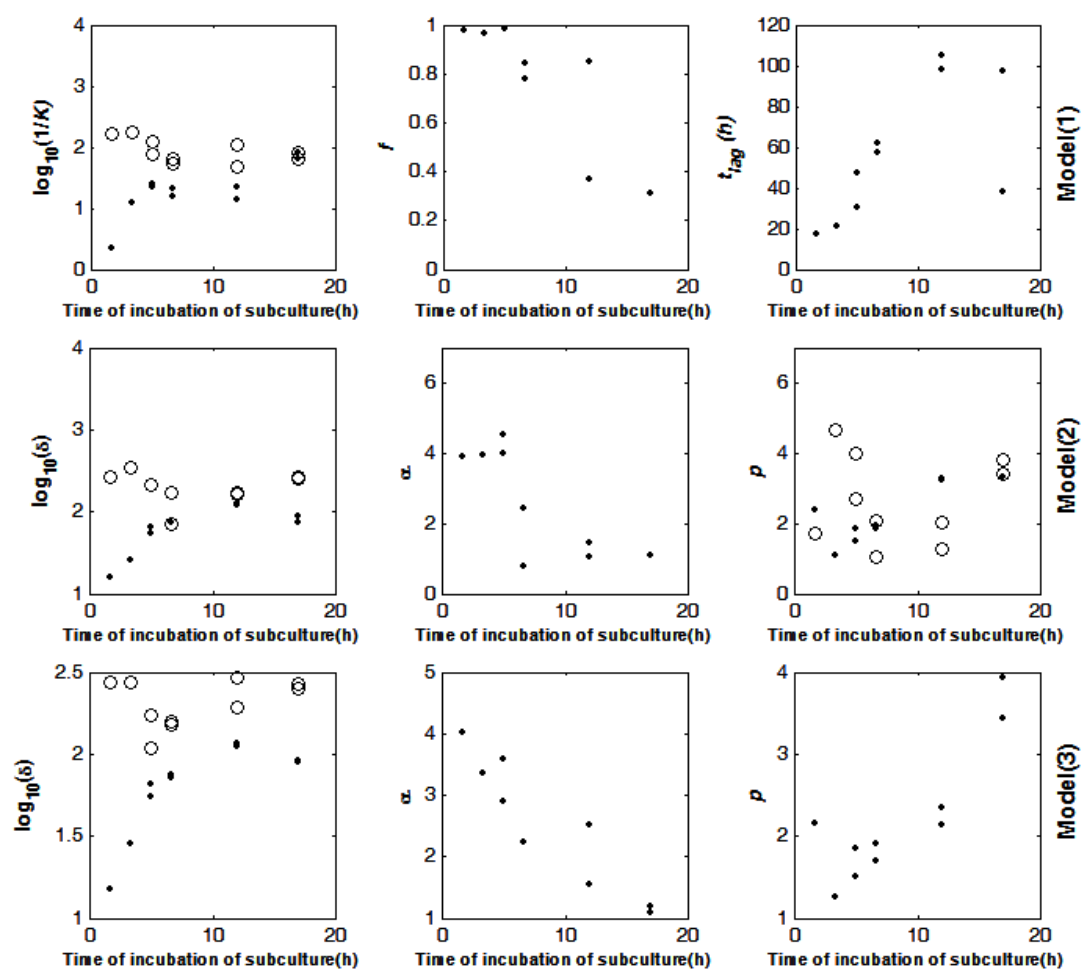


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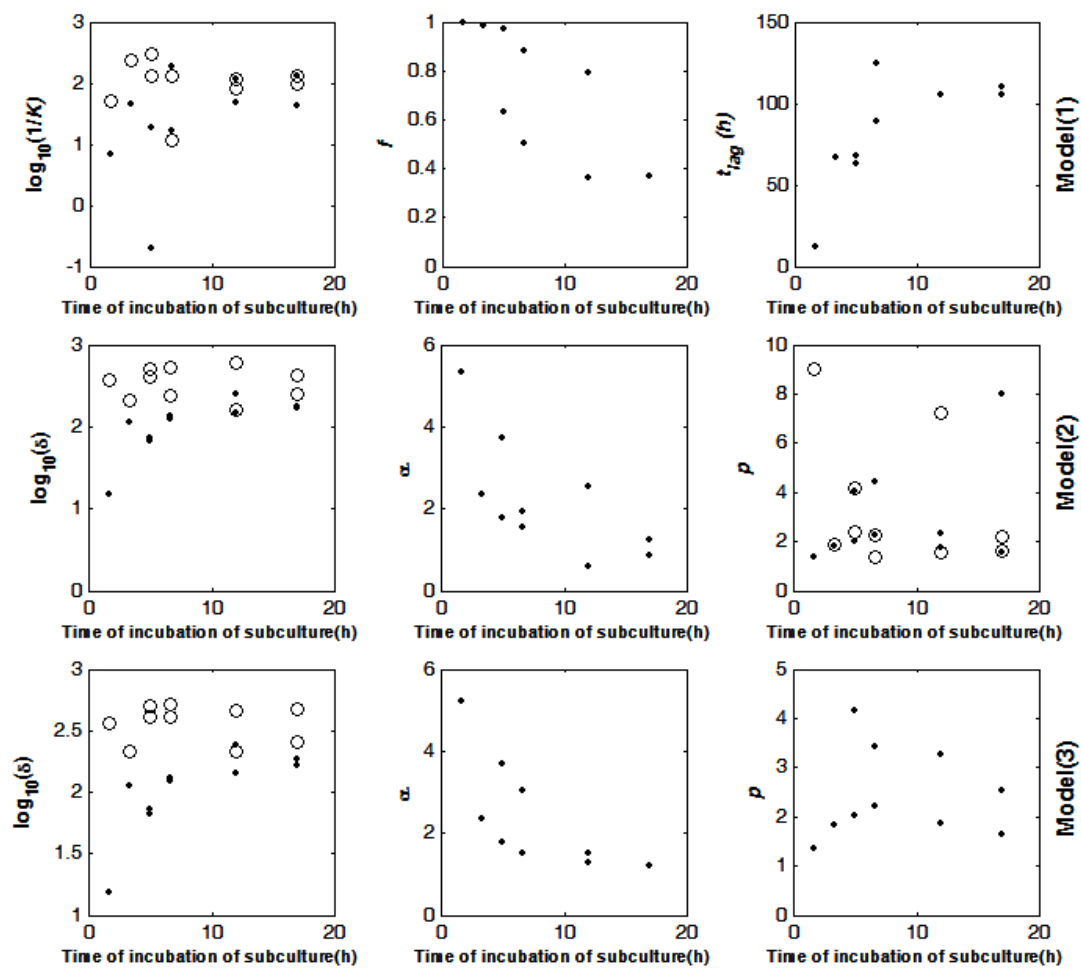


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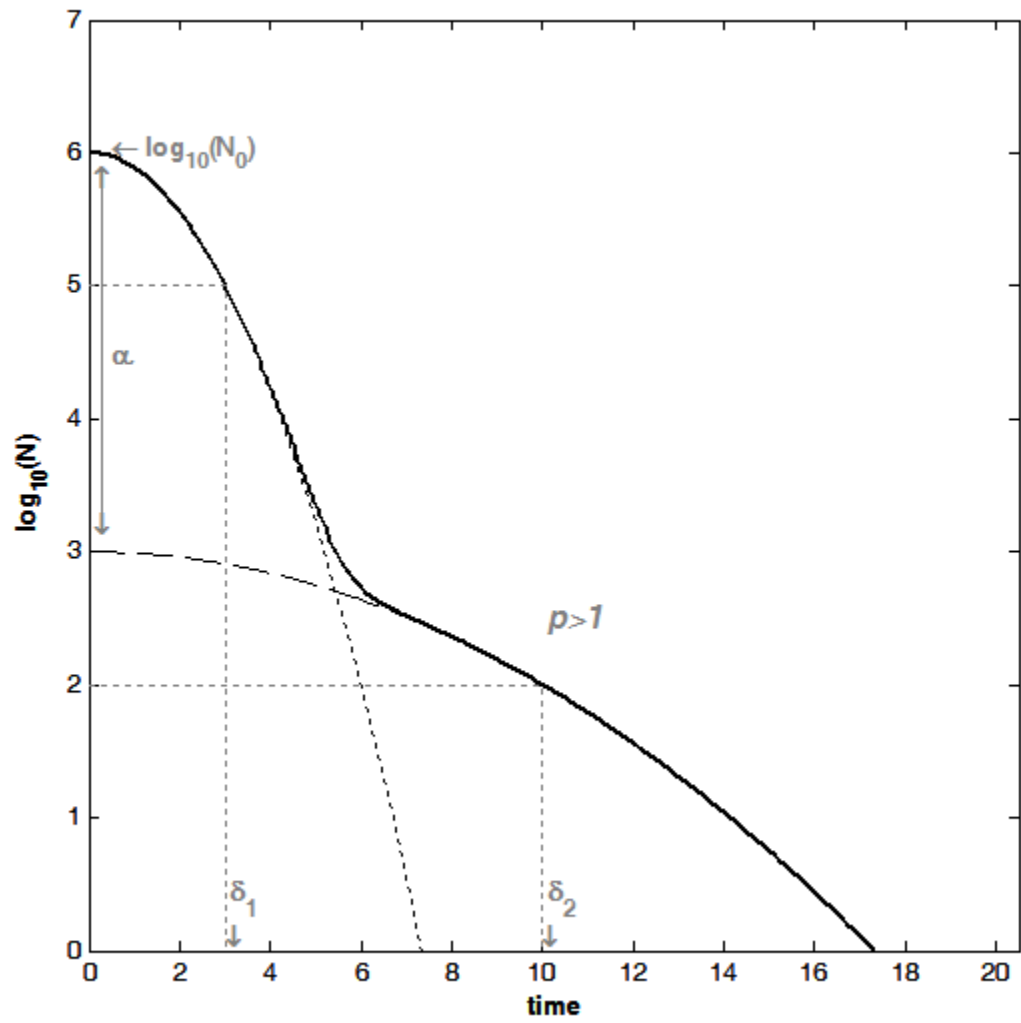


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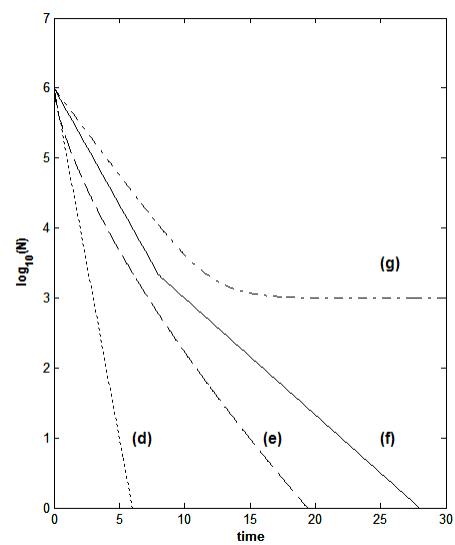
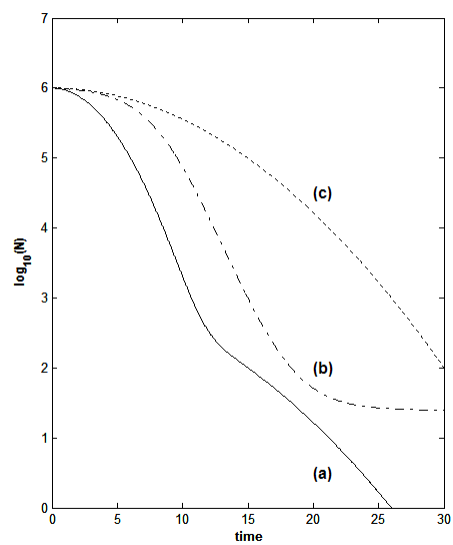


Figure 7